References of Record

Applicants have noticed the Examiner's comment that mere listing of references in the specification does not guarantee consideration of those references. The Examiner's comment that only references listed on a form PTO-890 are considered is in error however. Applicants submit that references listed on a form PTO-1449 submitted with an Information Disclosure Statement are also to be considered by the Examiner and listed on the front of any issued U.S. Patent as having been considered. Applicants note the filing of an Information Disclosure Statement in this application on August 11, 1998 and the return of the initialed form PTO-1449 with the pending Office Action. Applicants note that all of the citations have been initialed and expect that the Examiner has properly considered those references.

Claim Objections

Claim 12 was rejected as being in improper form. Applicants cancel claim 12, thereby rendering any objection to this claim moot.

Claim Rejections under 35 U.S.C. § 112

The Examiner has rejected claims 1-10 and 12-15 under 35 U.S.C. § 112, first paragraph for allegedly not being enabled by the specification. Applicants cancel claims 1-10 and 12-15, thereby rendering any rejection of these claims moot.

The Examiner argues that the instant specification does not provide enablement for functional analogues of the sakacin P gene cluster. Applicants respectfully disagree. The functional analogues of the promoters, genes, and peptides useful in the present invention are clearly defined in the present specification as being involved in the production of bacteriocins, except nisin, in lactic acid bacteria. For example, on page 7, lines 19-23, an analogue of the IF-K-R gene cluster is clearly defined as the *plnABCD* gene cluster from *Lactobacillus plantarum* C11. Furthermore, on page 10, lines 15-23, Applicants describe that by sequence comparison to regulatory promoter elements found in front of regulated genes involved in bacteriocin production relevant promoters can be identified. For example, elements in front of regulated genes involved in bacteriocin production by *Lactobacillus plantarum* C11 and *Staphylococcus aureus* have been identified by this method.

Example 2 of the specification (pages 14-15) describes how to purify and characterize a peptide inducing a bacteriocin. A skilled artisan in this technological field would be able to replace the bacteriocin with their gene of interest. Addition of the test peptide would cause expression of the gene of interest, which is assayed for by, for example, a Northern blot, an assay commonly known in the art. Furthermore, Example 4 of the specification (pages 16-18) describe the analysis of genes and promoters involved in the production of the sakacin P. One of ordinary skill in the art could use the same procedures to analyze genes, promoters, and inducing peptides involved in the production of other bacteriocins in another lactic acid bacteria. This assertion is supported by the attached recent publications describing the existence of functionally analogous systems of other lactic acid bacteria. Although the specification does not state which nucleic acid residues of the promoters or genes, or which amino acid residues of the peptides may be changed in order to retain their function, the specification does state that the functional analogues are identifiable as being similar in sequence, and their function may be assayed by a simple Northern blot procedure known in the art.

Exhibits 1-4 are papers by Diep et al., Quadri et al., Nilsen et al., and Axelssen et al., respectively, that show successful use of the approaches described in the specification to isolate functional analogs of the IF, SakK and SakR genes.

Finally, the Applicants wish to clarify two points raised by the Examiner. First, on page 6, lines 2-4, the Examiner states that the specification enables for an inducer that is sakacin P. Applicants wish to clarify that the inducer peptide ("unmodified peptide") is not sakacin P. The specification clearly states that the 19 residue unmodified peptide has a sequence identical or similar to that of the IF gene (SEQ ID NO:3).

Second, on page 5, lines 7-10, the Examiner argues that the specification does not teach the properties that distinguish the nisin gene from other promoters and peptides useful in the present invention. Applicants wish to clarify that the inducing peptides of the present invention differ fundamentally from nisin and its analogues, i.e. the lantibiotics. Nisin is a peptide with bacteriocin activity that also has a capacity to induce transcription of all genes necessary for its own production. Therefore, nisin is both the bacteriocin and the inducing peptide. However, the inducing peptide of the instant

invention in its natural setting induces production of another peptide that is the bacteriocin protein. Therefore, the inducing peptide and the bacteriocin to be induced are not the same peptide. Thus, nisin and the other lantibiotics are distinguished from the inducing peptide of the present invention in that the inducing peptide of the invention has little or no bacteriocin activity.

Further, the genetic organization of the nisin gene clusters and the gene clusters of the present invention are fundamentally different. Specifically, in its natural setting, the gene encoding nisin is not adjacent to or co-transcribed with genes encoding the cognate two-component regulatory system, as is the case for the IF genes of the present invention in their natural settings. New claim 41 recites that all of the IF gene, the SakK gene, and the SakR gene are components of the same operon. New claims 42 and 43 recite that the IF gene is in the same operon on the SakK and SakR genes, respectively.

Issues under 35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 1-10 and 12-15 under 35 U.S.C. § 122, second paragraph, as being indefinite. The Examiner's remarks have been considered and claims 1-10 and 12-

15 have been canceled. Therefore, rejections of these claims are rendered moot.

In the newly added claims the term "functional analogue thereof" is used. Applicants submit that this term is not indefinite as explained above. Nonetheless, the offending terms "unmodified peptide," "Seq ID," and "similar" are not used in the new claims. Furthermore, the newly added method claims recite positive action steps as suggested by the Examiner.

Issues under 35 U.S.C. § 101

The Examiner has rejected claims 12-14 under 35 U.S.C. § 101, for alleged lack of utility. The Examiner's remarks have been considered and claims 12-14 have been canceled. Therefore, rejections of these claims are rendered moot.

Issues under 35 U.S.C. § 102(b)

The Examiner has rejected claim 11 under 35 U.S.C. § 102(b) as being anticipated by Diep et al. (1994), or Tichaczek et. al. (1994). Applicants cancel claim 11, thus rendering rejection of this claim moot. The Examiner is hereby directed to new claim 28, directed to the subject matter of claim 11, but reciting different limitations.

Although claim 11 has been cancelled, the Applicants wish to clarify the components of the present expression system so the Examiner can appreciate some distinctions between the present invention and the references. For example, the sequence showed by Tichaczek et al. is a SakP gene sequence. SakP is the final expression product derived from a gene whose transcription is controlled by the IF-K-R regulatory system of the lactic acid bacterium. However, Tichaczek et al. does not disclose or suggest this regulation of SakP transcription. In other words, the function of the IF-K-R gene cluster is to cause expression of SakP. In the present invention, SakP is analogous to the polynucleotide of interest recited in claim 23.

Further, Applicants submit that Diep et al. (1994) fails to disclose several important aspects of the present invention that are discussed below.

Diep et al. (1994) had only found some sequence similarities in a cloned DNA fragment. They did not know or publish the function of plnA in the 1994 reference.

Also, Diep et al. (1994) erroneously ascribe bacteriocin activity to plantaricin A. Thus, Diep et al. describe plantaricin A as a protein like nisin having both bacteriocin and inducing activity. (See page 160, column 1, line 13, and

page 163, column 2, lines 36-44, respectively). In contrast, the inducing peptide encoded by IF has only inducing fucntion.

Thus, Diep et al. (1994) did not show or suggest the coupled regulatory mechanism for bacteriocin production discussed in example 1 of the specification and recited in e.g. claim 16. That is, Diep et al. did not disclose the interaction of the plA gene product (inducer peptide) and the gene products of plnBCD (modifiers of the inducer). The discovery of the inducing mechanism subsequently permitted identification and functional testing of the inducible promoters as well as an investigation into the inducing mechanism by the inventors.

Furthermore, the similarities between the Agr system and the plnABCD system that are discussed in the paper are not sufficient for a skilled artisan in the field to easily recognize the actual function of the elements of the plnABCD operon. Indeed, not much was even known about the Agr system at the beginning of 1994.

As Diep et al. (1994) do not reveal the functional coupling between the plnA gene product and the products of the plnABCD genes, the subject matter of the present claims is not anticipated by Diep et al. (1994).

Issues under 35 U.S.C. § 103

The Examiner has rejected claim 1-10 and 12-15 under 35 U.S.C. § 103 as being unpatentable over Diep et al. (1995). Applicants have been in contact with Blackwell Science Ltd. which publishes the journal *Molecular Microbiology*. The publication date of the Diep et al. reference is December 13, 1995, whereas the submission date of the instant patent application is November 13, 1995. Therefore, the publication of the Diep et al. (1995) article did not become publicly available prior to filing of the instant patent application. This fact is consistent with the International Search Report, wherein the Diep et al. (1995) publication is a category "P" (document published prior to the international filing date but later than the priority date claimed). Therefore, Diep et al. (1995) is not prior art and any rejection of the present claims as unpatentable over Diep et al. (1995) is overcome.

For all of the above reasons, Applicants respectfully submit that all of the present claims define patentable subject matter such that this application should be placed into condition for allowance. Early and favorable action of the merits of the present application is respectfully requested.

Pursuant to 37 CFR 1.17 and 1.136(a), the Applicants respectfully petition for a two (2) month extension of time for filing a response in connection with the present application and the required fee of $190.00 is attached hereto.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Kristi L. Rupert (Reg. No. P-45,702) at the telephone number below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and further replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fee required under 37 C.F.R. 1.16 or under 37 C.F.R. 1.17; particularly, extension of time fees.

Respectfully yours,

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Enclosures: Exhibit 1 - Diep, Dzung Bao et al., *Jour. of Bacteriology*, pp. 4472-4483, (August 1996)
Exhibit 2 - Nilsen, Trine et al. *Jour. of Bacteriology*, pp. 1848-1854, (April 1998)
Exhibit 3 - Quadri, Luis E. N. et al., *Jour. of Bacteriology*, pp. 6163-6171 (October 1997)
Exhibit 4 - Axelsson, Lars et al., *FEMS Microbiology Letters*, pp. 137-143, (1998)